The interplay of dietary nutrient specification and varying calcium to total phosphorus ratio on efficacy of a bacterial phytase: 1. Growth performance and tibia mineralization

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Abbreviated title: DIET FACTORS AND PHYTASE EFFECT ON GROWTH

ABSTRACT A 14-d experiment was conducted to study the effects of two dietary variables on efficacy of a 6-phytase from *Citrobacter braakii* on broiler growth performance and tibia mineralization. Diets were formulated with or without nutrient matrix values for phytase as negative or positive control (**NC** or **PC**, respectively) and with two Ca:total P, **tP** (2:1 or 2.5:1). The diets were supplemented with 0, 1,000 or 2,000 FYT/kg of phytase thus producing a 2×2×3 factorial arrangement. Birds and feed were weighed on days 7 and 21 and tibia bones were collected from all the birds on day 21. The main effects of nutrient matrix, Ca:tP and phytase supplementation were significant ($P < 0.05$) for all the growth performance responses (except for gain:feed for which there was no effect of matrix). Ca:tP × phytase and matrix × phytase interactions were significant ($P < 0.05$) for weight gain. In the PC diets, phytase increased weight gain ($P < 0.05$) relative to the control only in diets with 2,000 FYT/kg phytase, whereas in NC diets weight gain increased ($P < 0.01$) only from 0 to 1,000 FYT/kg phytase levels. Broilers consuming diets with 2.5:1 Ca:tP had lower ($P < 0.05$) tibia ash whereas phytase increased ($P < 0.01$) tibia ash, Ca, P, and Zn but decreased ($P < 0.01$) tibia K. Phytase supplementation of diets with 2:1 Ca:tP increased ($P < 0.05$) tibia P in birds receiving 1,000 FYT/kg relative to the control with no further increase at 2,000 FYT/kg whereas each level of phytase supplementation increased ($P < 0.05$) tibia P in the diets with 2.5:1 Ca:tP. It was concluded that the best response to lower phytase supplementation (1,000 FYT/kg) was in NC diets with narrow Ca:tP whereas the best response to higher level of phytase supplementation (2,000 FYT/kg) was achieved in diets in PC diets with wide Ca:tP.

Key words: broiler, calcium:phosphorus, growth, phytase matrix,

INTRODUCTION

The beneficial effects of phytase in diets marginally deficient in P and Ca and some amino acids have been widely reported in the literature (Olukosi et al., 2007; Selle and Ravindran, 2007; Adeola and Cowieson, 2011). Although phytase is supplemented primarily to liberate P from phytate and hence is a necessary additive in diets that are deficient in P and Ca, phytase have been beneficial as well in diets that are formulated to be adequate in P (Watson et al., 2006; Adeola and Cowieson, 2011). In addition, phytase supplementation at levels higher than 1,000 FTU/kg has been shown to be beneficial to birds in terms of growth performance response (Shirley and Edwards, 2003; Cowieson et al., 2006).

The negative effect of wide Ca:P on phytase efficacy is well known (Tamim et al., 2004; Adeola et al., 2006) and this is related to the formation of recalcitrant Ca-phytate (Taylor, 1965; Nelson and Kirby, 1987) or Ca-phosphate complexes (Long et al., 1984). Phytase is usually supplemented to diets that are deficient in P and hence most of the investigations of the effects of Ca: total P (**tP**) on phytase efficacy are done in P-deficient diets. Because there is increasing interest in phytase supplementation of nutrient-adequate diets (Walk et al., 2013), it is also vital to investigate the effect of widening Ca:tP on phytase efficacy in such diets.

Therefore the objective of the current experiment was to investigate how the use of a nutrient matrix values for a phytase, from *Citrobacter braakii*, affects the efficacy of the phytase on growth performance and tibia bone mineralization and especially within the context of variable dietary Ca:tP. The companion article considers how these dietary factors influence nutrient utilization in broilers.

MATERIALS AND METHODS

All the animal experimentation procedures used in the current study were approved by the Scotland's Rural College's Animal Experimentation Committee.

Diets and experimental design

A total of 576 day-old birds were used for the 14-d experiment to study the influence of nutrient specifications (use of nutrient matrix values for phytase) and Ca:tP on efficacy of phytase on growth performance and tibia mineralization. The birds were brooded together in a floor pen for the first 7 days during which they received a standard diet that meets NRC (1994) nutrient requirement for broilers. On day 7, the birds were weighed and allocated to 12 dietary treatments in a randomized complete block design and a 2×2×3 factorial arrangement of treatments. Each treatment had 8 replicate cages and 6 birds per replicate cage. The factors were two levels of nutrient specifications (explained below), two levels of Ca:tP (narrow, 2.1 and wide, 2.5:1) and three levels of phytase supplementation (0, 1,000 and 2,000 FYT/kg). Birds and feed were weighed on day 21 after which the birds were euthanized and the left tibia were collected from each of the birds.

The composition of the experimental diets is presented in Table 1. Nutrient specification was used to define the diets that were formulated to meet all the nutrient requirements for broilers (full nutrient specification without nutrient matrix phytase or positive control, **PC**) and another set of diets with reduced nutrient specification formulated to be deficient in P, Ca, CP, amino acids, and energy (down specification or negative control, **NC**). The nutrients and energy levels in the NC diets were reduced relative to the PC diets on the basis of the amount of nutrients and energy that the phytase was expected to release (nutrient matrix values for phytase). The matrix values used per kg feed for 1,000 FYT were approximately, 75 kcal ME, 1.5 g for available P, 1.8 g for Ca, 0.26 g for crude protein, 0.11, 0.07, 0.04, and 0.07 g for digestible Lys, total sulphur amino acids, Met, and Thr, respectively. One phytase (**FYT**) unit is defined as the activity that releases 1 μmol inorganic phosphate from 5.0 mM phytate per minute at pH 5.5 and 37°C.

Analyses

Chemical analysis. The tibia bones were first extracted with ethyl ether prior to being ashed in a muffle furnace and the ash analyzed for minerals. Bones were ashed for 24 hours at 600°C in a muffle furnace (Method 942.05; AOAC, 2006). Diets were analyzed for dry matter, N, gross energy, and minerals. Dry matter was determined by drying the samples in a drying oven (Uniterm, Russel-Lindsey Enginering Ltd., Birmingham, England, UK) at 100°C for 24 hours (Method 934.01; AOAC, 2006). Total N content was determined by the combustion method (Method 968.06; AOAC, 2006). Gross energy value was determined in an adiabatic oxygen bomb calorimeter (Model 6200, Parr Instruments, Moline, IL) using benzoic acid as standard. Mineral content was determined using Inductively Coupled Plasma – Optical Emission Spectroscopy (Method 990.08; AOAC, 2006) following digestion, in turn, in concentrated HNO_3 and HCl.

Statistical analysis. The data on growth performance and tibia bone mineralization were analyzed using the GLM procedure of SAS as appropriate for a factorial arrangement of treatments. When interactions were not significant, the interaction term was removed and the data re-analyzed to examine the main effects. Multiple regression analyses were performed using REG procedure of SAS to examine the effects of Ca:tP or nutrient matrix on response of birds to phytase supplementation. In all cases, significance was declared at $P \leq 0.05$.

RESULTS

The analyzed compositions of experimental diets are presented in Table 2. Overall the nutrient, energy and phytase contents were within target except in diet 3 where the phytase recovery was at 2,977 instead of the expected 2,000 FYT/kg. The levels of Ca were higher than expected but the levels of total and phytate P were within the expected levels. The effects of the dietary treatments on growth responses are shown in Table 3. The main effects of nutrient matrix (i.e. NC vs. PC), Ca:tP and phytase supplementation were significant ($P < 0.05$) for all the growth performance responses except for gain:feed for which there were no significant matrix and Ca:tP effects. Weight gain and feed intake were lower ($P < 0.05$) in NC compared with PC diets as well as in the diets with wide Ca:tP compared with the diets with narrow Ca:tP. There was no matrix × Ca:tP interaction for any of the growth performance responses; matrix × phytase and Ca:tP × phytase interactions were only significant ($P < 0.05$) for weight gain. In PC diets, phytase increased weight gain relative to the control only at 2,000 FYT/kg whereas in NC diets weight gain increased ($P < 0.01$) dramatically in diets with 1,000 FYT/kg compared to the control and only marginally increased (numerically only) further with 2,000 FYT/kg. In addition, in the diets with 2:1 Ca:tP, phytase supplementation at 1,000 FYT/kg increased weight gain ($P < 0.05$) relative to the control but no further increase was observed at 2,000 FYT/kg whereas phytase supplementation produced a stepwise increase ($P < 0.05$) in weight gain in the diets with 2.5:1 Ca:tP.

The tibia bone mineralization responses to the dietary treatments are shown in Table 4. Tibia ash, Ca and P were lower ($P < 0.01$) in NC relative to PC diets. Birds consuming diets with 2.5:1 Ca:tP had lower tibia ash and Mg whereas phytase supplementation increased ($P < 0.01$) tibia ash, Ca and Mg. The two-way interactions were largely not significant for most of the tibia mineralization responses except for Ca:tP × phytase effect ($P < 0.05$) for tibia P and matrix × phytase interaction ($P < 0.05$) for tibia K and Zn. Phytase supplementation of diets with 2:1 Ca:tP increased ($P < 0.05$) tibia P in diets with 1,000 FYT/kg relative to the control with no further response at 2,000 FYT/kg whereas each level of phytase supplementation increased ($P < 0.05$) tibia P in the diets with 2.5:1 Ca:tP. In addition, phytase supplementation had no effect on tibia K in the PC diets but tibia K was lower ($P < 0.05$) in NC diets supplemented with phytase.

DISCUSSION

The objective of the current study was to investigate the interactivity of Ca:tP and dietary nutrient content or matrix (PC vs. NC) on phytase supplementation effects on growth performance and tibia mineralization. In view of the importance of P for bone development, and ultimately, bird growth, the diets were formulated to be either limiting or sufficient in P and hence the birds were expected to respond rapidly to phytase supplementation.

As expected growth performance responses were greater in the un-supplemented PC compared with the un-supplemented NC and this can be directly related to nutrient content of the diets. The PC diets were formulated to meet nutritional requirements of the birds whereas the NC diets were not. Response to dietary manipulation ultimately depends on the supply of the nutrients limiting for growth (Olukosi et al., 2007). In the PC diets, the limiting nutrient (i.e. P in this experiment) was supplied in the diets as inorganic P whereas in the NC diets, P was expected to be released from hydrolysis of phytic acid by the supplemented phytase. Consequently when phytase improves growth performance of birds in diets not limiting in P, this effect can only be attributed to other factors apart from P (Cabahug et al., 1999; Walk et al., 2013). Such factors could be the release of other nutrients or minerals that may otherwise have been deficient (Keshavarz, 2000) or by means of modified digesta viscosity (Watson et al., 2006)

The improvement in weight gain was 68% greater in NC diets containing 2,000 FYT/kg phytase compared with the same level of phytase supplementation in PC diets. Phytase at 1,000 FYT/kg was sufficient to completely reverse the negative effects on weight gain produced by the low level of non-phytate P in the NC diets and produced weight gain that was numerically greater than that observed in PC diet without phytase. Supplementation of additional 1,000 FYT/kg phytase produced only marginal improvement in weight gain in the NC diet and hence in the present study 1,000 FYT/kg was sufficient to produce optimum weight gain response in the NC diet.

Unlike weight gain, the increase in bone mineralization in response to phytase supplementation was independent of nutrient matrix. The interaction of nutrient matrix (i.e. PC or NC) and phytase supplemental level on weight gain can therefore be disassociated from the effect of these factors on bone mineralization. The rates of weight gain and bone development in birds are directly related to some extent (Leterrier and Nys, 1992; Williams et al., 2004) and several studies with phytase have simultaneously investigated its effects on weight gain and bone mineralization (Onyango et al., 2004; Olukosi et al., 2013). However as shown in the current study, weight gain and bone mineralization do not respond the same way to dietary manipulations likely because bone mineralization depends to a greater extent on P and Ca supply than weight gain. This likely account for why phosphorus equivalency is different when calculated using weight gain or bone ash (Yi et al., 1996; Adedokun et al., 2004)

Three inferences are possible from the observation of the effects of phytase on weight gain in PC and NC diets. First, because the weight gain in PC diet without phytase was 17%

greater than the weight gain in NC diet without phytase, the PC diet provided "less room" or "opportunity" for phytase effect on weight gain. In a situation where P is presumably no longer the limiting nutrient for growth, it would require higher levels of phytase supplementation to produce an effect. This may be one of the reasons why Walk et al. (2013) did not observe a weight gain response to phytase supplementation of the P-adequate diet in their study. Secondly, because the PC diet was formulated to meet all nutrients requirement and the NC diets were formulated to be limiting in P, the improvement in growth performance observed at 2,000 FYT/kg in the PC diet could be due to other beneficial effects of phytase beyond P release (Cabahug et al., 1999; Walk et al., 2013).

The third inference relates to the magnitude of response to phytase supplementation produced by the additional 1,000 FYT/kg phytase in PC and NC diets (i.e. the 2,000 FYT/kg diets). Beyond the 1,000 FYT/kg phytase level, every additional 100 FYT/kg produced 6.5 g of weight gain in PC diet but only 2 g of weight gain in the NC diet. Because the PC diet met all the nutrient requirements and NC did not, and also because weight gain produced by 1,000 FYT/kg phytase was the same for both NC and PC, the comparatively lower weight gain response to phytase supplementation in the NC diet beyond 1,000 FYT is possibly an indication that higher rate of phytase supplementation to NC diet may be hampered by factors not yet understood. For example, it could offset Ca:P ratio or produce other nutrient imbalances which may negatively influence performance. In contrast this effect was not observed in PC diet, in which case it is possible that the totality of the available nutrient levels in the diets and nutrients released by phytase were in performance-conforming balance. In fact, the ratio of ileal digestible Ca to digestible tP was consistently wider in NC compared with PC diets irrespective of phytase supplementation (reporting in companion article).

The impact of Ca:P on phytase efficacy using growth performance is well known (Qian et al., 1997; Delezie et al., 2012). As shown in the companion article, the negative effect of increasing Ca:tP was more severe in the NC diets. However, there were no interaction between nutrient matrix and Ca:tP on growth and bone mineralization responses in the current study. On the other hand, efficacy of phytase in improving body weight gain and tibia mineralization (tibia content of P and Ca) depended on Ca:tP. In the diets with narrow Ca:tP, phytase efficacy was only apparent between the 0 and 1,000 FYT/kg. On the other hand, in the diets with wider Ca:tP there was a step-wise increase in weight gain. The improvement in weight gain produced by 2,000 FYT/kg phytase (relative to 0 FYT/kg) in the diets with wide Ca:tP was 250% of that produced in the diet with narrow Ca:tP. There was no difference in weight gain at 2,000 FYT/kg phytase level in the diets with wide or narrow Ca:tP but in the diets without phytase, weight gain in the diets with narrow Ca:tP was 14% greater than the weight gain in diets with wide Ca:tP. Consequently, phytase effect was muted in the diets with narrow Ca:tP than in the diets with wide Ca:tP. This is presumably because the diets with narrow Ca:tP had more readily available P (the limiting mineral for growth in the current study) and hence the phytase effect was less apparent in these diets.

Further calculations of Ca and P intake as well as retained Ca and P in relation to dietary Ca:tP can help explain some of the effects of Ca:tP observed on growth performance. The ratios of intakes of Ca and P followed dietary Ca to tP ratios. However, the ratio of retained Ca:tP (1.4:1 for diets with Ca:tP of 2:1 and 1.1:1 for diets with 2.5:1, respectively) was narrower and reversed compared with the dietary and intake ratios of Ca:tP. These reversals were because the retained Ca and P were greater for diets with narrow Ca:tP which is likely an indication of greater availability of these minerals (possibly due to less Ca-phosphate complex formation) in the diets with narrow Ca:tP.

When the observations of the weight gain responses to low and high phytase supplemental levels are taken together, two conclusions are apparent. One conclusion is that 1,000 FYT/kg is sufficient to offset the initial depressing effect on weight gain of the low dietary non-phytate P in the current study. The second conclusion is that higher level of phytase supplementation is needed in the diets containing wide Ca:tP to more fully reverse the negative impact of wide Ca:tP. Because the diets with wide Ca:tP had higher concentration of Ca relative to P, it can be reasoned that the use of phytase up to 1,000 FYT/kg released more P and hence possibly helped to offset the negative consequence of the imbalance in Ca:tP. Phytase supplementation is also expected to increase P release in diets with narrow Ca:tP, but in this case because the Ca:tP ratio has always been more favorable, increased P release may not produce a very noticeable effect. Or it could be that increased P concentration relative to Ca, increases the chances for formation of calcium-phosphate (Long et al., 1984) thus reducing both the absorption of Ca and weight gain (Masuyama et al., 2003).

Phytase is routinely added at the rates of between 500 and 1,000 phytase units per kilogram however the use of higher levels of phytase supplementation has been reported to provide greater response than observed at the lower supplementation level and some of these positive effects are not necessarily due to increased P release (Shirley and Edwards, 2003; Cowieson et al., 2006; Cowieson et al., 2011). The current study shows that phytase supplementation up to 2,000 FYT/kg is more advantageous in the PC diets and in the diets with wide Ca:tP. The observation that the high phytase level was not more effective than 1,000 FYT/kg in the NC, in contrast to PC, diets is an indication that the additional benefit observed beyond 1,000 FYT/kg level was not due to increased P supply as also suggested by Walk et al. (2013). It is reasonable to assume that there is a limit to phosphate uptake from the lumen of birds and hence greater P availability will not necessarily produce greater performance response once the requirement for P is met (Yan et al., 2003). The observation therefore that high phytase supplemental level was more efficacious in diets already meeting P requirement is a demonstration of the extra-phosphoric effect of phytase. On the other hand, in the diets with narrow Ca:tP it might be necessary to understand the nutrient imbalances that could results from the use of phytase to optimize growth.

Taken together, the data from the current study showed that the best response to lower phytase supplementation is in diets with narrow Ca:tP combined with reduced nutrient specification (NC) whereas the best response to high level of phytase supplementation is achieved in diets with full nutrient specification (PC) and having wider Ca:tP.

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REFERENCES

Adedokun, S. A., J. S. Sands, and O. Adeola. 2004. Determining the equivalent phosphorus released by an Escherichia coli-derived phytase in broiler chicks. Can. J. Anim. Sci. 84:437-444.

Adeola, O., and A. J. Cowieson. 2011. Opportunities and challenges in using exogenous enzymes to improve non ruminant animal production. J. Anim. Sci. 89:3189-3218.

Adeola, O., O. A. Olukosi, J. A. Jendza, R. N. Dilger, and M. R. Bedford. 2006. Response of growing pigs to *Peniophora lycii*- and *Escherichia coli*-derived phytases or varying ratios of calcium to total phosphorus. Anim. Sci. 82:637-644.

Cabahug, S., V. Ravindran, P. H. Selle, and W. L. Bryden. 1999. Response of broiler chickens to microbial phytase supplementation as influenced by dietary phytic acid and non-phytate phosphorus contents. I. Effects on bird performance and toe ash. Br. Poult. Sci. 40:660-666.

Cowieson, A. J., P. Wilcock, and M. R. Bedford. 2011. Super-dosing effects of phytase in poultry and other monogastrics. World's Poult. Sci. J. 67:225-235.

Cowieson, A. J., T. Acamovic, and M .R. Bedford. 2006. Supplementation of corn-soy-based diets with an *Eschericia coli*-derived phytase: effects of broiler chick performance and the digestiblity of amino acids and metabolizability of minerals and energy. Poult. Sci. 85:1389-1397.

Delezie, E., L. Maertens, and G. Huyghebaert. 2012. Consequences of phosphorus interactions with calcium, phytase, and cholecalciferol on zootechnical performance and mineral retention in broiler chickens. Poult. Sci. 91:2523–2531.

Keshavarz, K. 2000. Reevaluation of nonphytate phosphorus requirement of growing pullets with and without phytase. Poult. Sci. 79:1143-1153.

Leterrier, C., and Y. Nys. 1992. Composition, cortical structure and mechanical properties of chicken tibiotarsi: effect of growth rate. Br. Poult. Sci. 33:925-939.

Long, P. H., S. R. Lee, G. N. Rowland, and W. M. Britton. 1984. Experimental rickets in broilers: gross, microscopic, and radiographic lesions. I. phosphorus deficiency and calcium excess. Avian Dis. 28:460-474.

Masuyama, R., Y. Nakaya, S. Katsumata, Y. Kajita, M. Uehara, S. Tanaka, A. Sakai, S. Kato, T. Nakamura, and K. Suzuki. 2003. Dietary calcium and phosphorus ratio regulates bone mineralization and turnover in vitamin D receptor knockout mice by affecting intestinal calcium and phosphorus absorption. J. Bone Min. Res. 18:1217-1226.

Nelson, T. S., and L. K. Kirby. 1987. The calcium binding properties of natural phytate in chick diets. Nutr. Rep. Int. 35:949–956.

Olukosi, O. A., C. Kong, F. Fru-Nji, K. M. Ajuwon, and O. Adeola. 2013. Assessment of a bacterial 6-phytase in the diets of broiler chickens. Poult. Sci. 92:2101-2108.

Olukosi, O.A., A. J. Cowieson, and O. Adeola. 2007. Age-related influence of a cocktail of xylanase, amylase and protease or phytase individually or in combination in broilers. Poult. Sci 86:77-86.

Onyango, E. M., M. R. Bedford, and O. Adeola. 2004. The yeast production system in which *Escherichia coli* phytase is expressed may affect growth performance, bone ash, and nutrient use in broiler chicks. Poult. Sci. 83:421-427.

Qian, H., E. T. Kornegay, and D. M. Denbow. 1997. Utilization of phytate phosphorus and calcium as influenced by microbial phytase, cholecalciferol, and the calcium:total phosphorus ratio in broiler diets. Poult. Sci. 76:37-46.

Selle, P. H., and V. Ravindran. 2007. Microbial phytase in poultry nutrition. Anim. Feed Sci. Technol. 135:1-41.

Shirley, R. B. and H. M. Edwards Jr. 2003. Graded levels of phytase past industry standards improves broiler performance. Poult. Sci. 82:671-680.

Tamim, N. M., R. Angel, and M. Christman. 2004. Influence of dietary calcium and phytase on phytate phosphorus hydrolysis in broiler chickens. Poult. Sci. 83:1358-1367.

Taylor, T. G. 1965. The availability of the calcium and phosphorus of plant materials for animals. Proc. Nutr. Soc. 24:105–112.

Walk, C. L., M. R. Bedford, T. S. Santos, D. Paiva, J. R. Bradley, H. Wladecki,C. Honaker, and A. P. McElroy. 2013. Extra-phosphoric effects of superdoses of a novel microbial phytase. Poult. Sci. 92:719-725.

Watson, B. C., J. O. Matthews, L. L. Southern, and J. L. Shelton. 2006. The effects of phytase on growth performance and intestinal transit time of broilers fed nutritionally adequate diets and diets deficient in calcium and phosphorus. Poult. Sci. 85:493–497.

Williams, B., D. Waddington, D. H. Murray, and C. Farquharson. 2004. Bone strength during growth: influence of growth rate on cortical porosity and mineralization. Calcif. Tissue Int. 74:236–245.

Yan, F., J. H. Kersey, C. A. Fritts, and P. W. Waldroup. 2003. Phosphorus requirements of broiler chicks six to nine weeks of age as influenced by phytase supplementation. Poult. Sci. 82:294–300.

Yi, Z., E. T. Kornegay, V. Ravindran, and D. M. Denbow. 1996. Improving phytate phosphorus availability in corn and soybean meal for broilers using microbial phytase and calculation of phosphorus equivalency values for phytase. Poult. Sci. 75:240-249.

Table 1. Ingredient composition of the experimental basal diets

Basal diets	1	2	3	4
Ca:tP	2:1		2.5:1	
Control (phytase matrix)	Positive	Negative	Positive	Negative
Corn	482.6	477.4	466.6	499.4
Wheat	-	50.0	-	-
Soybean meal	397.5	382.5	400.5	394.5
Soybean oil	58.0	40.0	60.0	45.0
Corn starch	15.0	15.0	15.0	15.0
Dicalcium phosphate	17.5	9.0	17.5	10.0
Limestone	17.0	15.5	28.0	24.0
Titanium dioxide	0.5	0.5	0.5	0.5
L-Lysine·HCl	1.0	0.4	1.0	0.7
DL-Methionine	2.8	1.9	2.8	2.8
Threonine	0.6	0.3	0.6	0.6
Vitamin-mineral premix[1]	2.5	2.5	2.5	2.5
Salt	5.0	5.0	5.0	5.0
Phytase premix[2]	To 1000	To 1000	To 1000	To 1000
Total	1000	1000	1000	1000

[1]Supplied the following per kilogram of diet: vitamin A, 5,484 IU; vitamin D3, 2,643 ICU; vitamin E, 11 IU; menadione sodium bisulfite, 4.38 mg;riboflavin, 5.49 mg; d-pantothenic acid, 11 mg; niacin, 44.1 mg; choline chloride, 771 mg; vitamin B12, 13.2 µg; biotin, 55.2 µg; thiamine mononitrate,2.2 mg; folic acid, 990 µg; pyridoxine hydrochloride, 3.3 mg; I, 1.11 mg; Mn, 66.06 mg; Cu, 4.44 mg; Fe, 44.1 mg; Zn, 44.1 mg; Se, 300 µg.

[2]Phytase premix containing 200 phytase units (FYT)/g replaced corn starch to provide 1,000 or 2,000 FYT/kg.

Table 2. Analyzed nutrient composition (%, dry matter basis) and phytase activity in the experimental diets

Diets	1	2	3	4	5	6	7	8	9	10	11	12
Ca:tP	2:1						2.5:1					
Matrix	Positive Control			Negative Control			Positive Control			Negative Control		
GE, kcal/kg	4,452	4,681	4,652	4,672	4,619	4,603	5,720	5,782	4,610	4,641	4,572	4,580
Ether extract	10.2	10.3	8.8	7.11	7.83	6.84	11.4	11.3	8.94	7.67	8.26	7.95
Acid hydrolysed fat	11.1	9.49	9.80	8.03	7.96	8.03	12.1	12.9	9.70	8.30	9.37	8.37
N	3.99	3.86	4.13	3.84	3.90	3.80	5.11	4.70	4.15	4.04	3.73	3.88
Ca	1.37	1.63	1.40	1.31	1.18	1.17	2.23	2.24	1.87	1.06	1.47	1.38
P	0.73	0.81	0.71	0.65	0.61	0.57	0.93	0.91	0.74	0.48	0.60	0.62
Phytate P	0.26	0.24	0.23	0.25	0.23	0.24	0.28	0.28	0.29	0.24	0.23	0.29
Non-phytate P[1]	0.47	0.57	0.48	0.40	0.38	0.33	0.65	0.63	0.45	0.24	0.37	0.33
Na	0.23	0.26	0.24	0.26	0.25	0.23	0.24	0.28	0.23	0.17	0.23	0.19
Mg	0.18	0.18	0.16	0.18	0.18	0.16	0.23	0.22	0.18	0.15	0.18	0.19
Cu, mg/kg	11.3	15.8	14.7	12.5	10.2	10.2	28.2	18.2	12.4	10.2	13.6	15.8
Fe, mg/kg	82.4	87.0	79.2	90.7	78.5	71.3	111.3	106.5	83.3	56.6	81.4	79.0
Mn, mg/kg	81.3	94.9	87.1	100.9	84.2	83.8	107.1	105.1	83.3	61.1	83.7	86.9
Zn, mg/kg	79.1	96	99.5	92.9	84.2	81.5	101.4	99.5	83.3	58.9	75.7	79
K	1.16	1.14	1.00	1.16	1.19	0.96	1.47	1.43	1.14	0.97	1.15	1.28
Phytase, FTY/kg	BD[2]	1121	2977	BD	1406	2400	BD	1,717	2,537	BD	1011	2640

[1]Non-phytate phosphorus level was determined by subtracting phytate P from total P

[2]BD = below detection limit

Table 3. Growth performance response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

Ca:tP	2:1						2.5:1							P-values for main effects and interactions					
Matrix (M)[2]	Positive Control			Negative Control			Positive Control			Negative Control				Main effects			Interactions[3]		
Phytase (Ph)	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	SEM	M	Ca:tP	Ph	1	2	3
WG[4], g	807	836	838	690	786	809	709	744	873	605	799	816	26.1	0.001	0.017	0.001	0.34	0.011	0.013
FI[4], kg	1.07	1.13	1.13	0.99	1.08	1.08	0.98	1.01	1.12	0.90	1.06	1.09	0.003	0.010	0.001	0.001	0.21	0.051	0.051
G:F, g/kg	757	742	743	696	727	747	721	741	781	678	752	751	20.5	0.065	0.87	0.015	0.88	0.20	0.22

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage.

[2]Matrix = nutrient matrix for phytase, positive control (full nutrient specification) and negative control (reduced nutrient specification).

[3]Interactions: 1 (matrix × Ca:tP); 2 (matrix × phytase); 3 (Ca:tP × phytase); the three-way interaction was not significant for any of the responses.

[4]WG = weight gain; FI = feed intake.

Table 4. Tibia bone mineralization (%) response to varying levels of phytase supplementation, dietary Ca:total P broiler diets with or without nutrient matrix values for phytase[1]

Ca:tP	2:1						2.5:1							P-values for main effects and interactions					
Matrix (M)[2]	Positive Control			Negative Control			Positive Control			Negative Control				Main effects			Interactions[3]		
Phytase (Ph)	0	1000	2000	0	1000	2000	0	1000	2000	0	1000	2000	SEM	M	Ca:tP	Ph	1	2	3
Ash	50.1	50.7	52.0	47.8	49.4	51.1	47.6	49.5	50.8	44.9	48.9	50.8	0.78	0.005	0.002	0.001	0.65	0.18	0.16
P	8.11	8.28	8.51	7.55	8.08	8.10	7.51	7.98	8.41	6.91	7.75	8.21	0.16	0.001	0.001	0.001	0.80	0.26	0.024
Ca	17.1	17.5	17.8	16.4	17.1	17.1	16.4	17.0	17.9	15.4	16.6	17.6	0.33	0.003	0.081	0.001	0.80	0.60	0.058
Mg	0.32	0.34	0.34	0.30	0.32	0.34	0.28	0.31	0.33	0.27	0.30	0.31	0.01	0.008	0.001	0.001	0.79	0.88	0.29
K	0.38	0.38	0.37	0.41	0.38	0.38	0.38	0.37	0.38	0.41	0.38	0.37	0.01	0.032	0.98	0.001	0.94	0.035	0.91
Zn[4]	147	174	185	162	175	191	138	174	191	159	178	186	4.30	0.006	0.58	0.001	0.75	0.007	0.46

[1]Means were obtained from 8 replicate cages of 6 birds per replicate cage.

[2]Matrix = nutrient matrix for phytase, positive control (full nutrient specification) and negative control (reduced nutrient specification).

[3]Interactions: 1 (matrix × Ca:tP); 2 (matrix × phytase); 3 (Ca:tP × phytase); the three-way interaction was not significant for any of the responses.

[4]Zn = unit is mg/kg